

CLAIMS

✓ 1. Method to produce a blank biochip, characterised in that it comprises the following steps:

a) structuring of a substrate so as to obtain on said substrate microtroughs comprising in their base a layer of a material capable of initiating and promoting the adhesion onto said layer of a film of a pyrrole and functionalised pyrrole copolymer by electropolymerisation,

b) collective electropolymerisation, so as to form an electropolymerised film of a pyrrole and functionalised pyrrole copolymer on the base of said microtroughs, on the layer of said material, using a pyrrole and functionalised pyrrole solution, in the presence of suitable chemical reagents for said electropolymerisation.

2. Method to produce a biochip comprising a blank biochip according to steps a) and b) of claim 1 and also comprising a step c) of direct or indirect fixation of a biological probe onto the functionalised pyrrole, by injecting a biological probe solution, either in one or more microtroughs in the presence of chemical reagents required for the direct or indirect fixation of this biological probe onto the functionalised pyrrole.

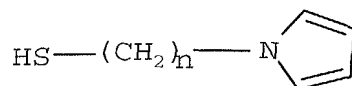
3. Method according to claim 1, wherein the layer of material capable of initiating and promoting the adhesion of the polypyrrole film by

electropolymerisation being a metallic layer, step a) comprises a deposition step of said metallic layer onto the substrate, and a deposition step of a layer of resin or polymer onto the metallic layer and engraving  
 5 of said resin layer so as to form microtroughs, wherein the base is composed at least partly of the metallic layer.

4. Method according to claim 3, wherein the  
 10 metallic layer is a gold layer.

*A* 5. Method according to claim 3 or 4, wherein step a) also comprises a chemical treatment step of the gold layer at the base of the microtroughs in the presence  
 15 of a functionalised pyrrole for example with a thiol group so as to form a monolayer of pyrrole onto said gold layer, at the base of said microtroughs.

6. Method according to claim 5, wherein the  
 20 functionalised pyrrole with a thiol group has the following chemical formula:



wherein n has a value ranging from 2 to 10.

*A* 7. Method according to ~~any of claims 1 to 6~~,  
 25 wherein the substrate is a silicon insert.

8. Method according to claim 1, wherein the substrate is a silicon insert and the layer capable of initiating and promoting the adhesion onto said layer

of the polypyrrole film by electropolymerisation being a layer of silane comprising an alignment of pyrrole sites, step a) comprises a deposition step of a layer of resin on the silicon insert, said silicon insert  
5 being coated with an  $\text{SiO}_2$  film, and engraving of said resin layer so as to form the microtroughs wherein the base is composed at least partly of the  $\text{SiO}_2$  film; and a microtrough treatment step by means of a functionalised silanisation agent with a pyrrole so as  
10 to fix, on the  $\text{SiO}_2$  film, in the base of the microtroughs, the silane layer comprising an alignment of pyrrole sites.

9. Method according to claim 8, wherein the  
15 silanisation agent is chosen in a group comprising N-(3-(trimethoxy silyl) propyl) pyrrole, or any other functionalised pyrrole with an  $-\text{SiCl}_3$  or  $-\text{Si}(\text{OMe})_3$  group.

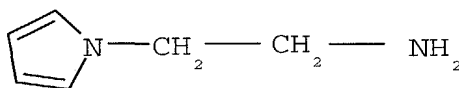
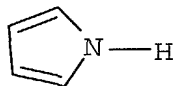
20 10. Method according to ~~any of claims 1 to 9~~, wherein the collective electropolymerisation is carried out by immersing the structured substrate obtained in step a) mentioned above in an electrolytic bath comprising a solution of pyrrole, functionalised  
25 pyrrole, and suitable chemical reagents for electropolymerisation, in the presence of a counter-electrode which is immersed in the electrolytic bath and is independent of the structured substrate, the layer of material capable of initiating and promoting  
30 the adhesion onto said layer of the pyrrole and

functionalised pyrrole copolymer film forming a working electrode.

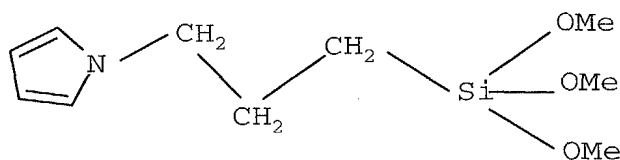
11. Method according to ~~any of claims 1 to 10~~,  
 5 wherein the functionalised pyrrole is a pyrrole comprising a group chosen in a set comprising an  $\text{NH}_2$  group, a thiol group, an N-hydroxysuccinimide ester group, a trimethoxy silyl group, a carboxyl, aldehyde and isothiocyanate group.

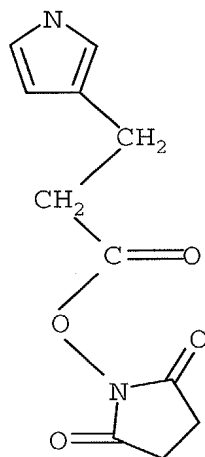
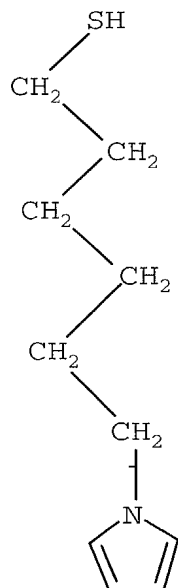
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12. Method according to ~~any of claims 1 to 10~~  
 wherein the functionalised pyrrole is chosen from one of the following compounds:



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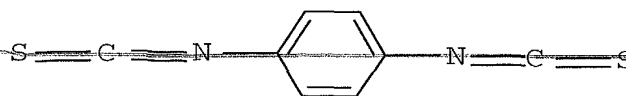
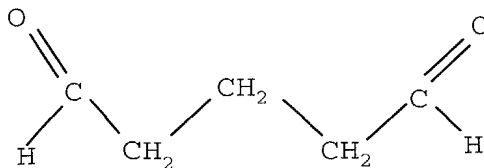
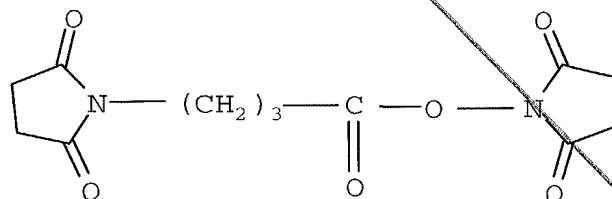
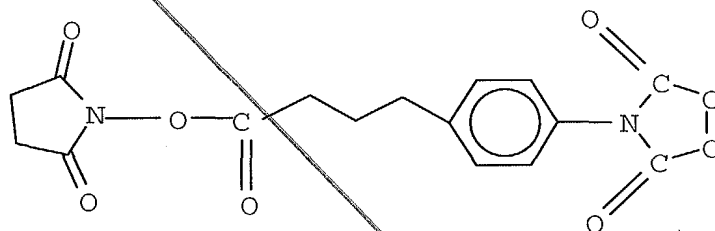


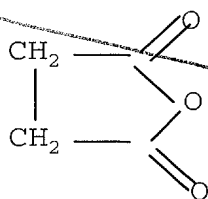
13. Method according to claim 2 wherein the fixation of the biological probe being indirect, said method also comprises, in step c); before the fixation of the biological probe, a collective fixation of a cross-linking agent on the functionalised pyrrole, in the presence of suitable chemical reagents, said cross-linking agent comprising a first function enabling its fixation onto the functionalised pyrrole, and a second

function enabling the fixation of the biological probe on said cross-linking agent.

14. Method according to claim 13, wherein the cross-linking agent is chosen in a set comprising a dialdehyde, diisothiocyanate, a diacid, a succinic anhydride, or a derivative of these compounds.

15. Method according to claim 13, wherein the cross-linking agent is chosen from one of the following compounds:





16. Method according to claim 2, wherein the biological probe is chosen from an oligonucleotide, a DNA, an RNA, a peptide, a glucide, a lipid, a protein, an antibody, an antigen.

17. Method according to claim 2, wherein the biological probe is a functionalised oligonucleotide to be fixed either directly or indirectly onto a functionalised pyrrole.

18. Method according to claim 17, wherein the oligonucleotide is functionalised with a thiol group.

15 ~~19. Blank biochip comprising in this order:~~

- a substrate,
- a layer of material capable of initiating and promoting on said layer the adhesion of a film of a pyrrole and functionalised pyrrole copolymer by electropolymerisation,
- a layer of resin coating said layer of material capable of initiating and promoting the adhesion on said layer of a film of a pyrrole and functionalised pyrrole copolymer, wherein microtroughs have been produced such that the base of said microtroughs is composed at least partly of the layer of said material,

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~~- a layer of a pyrrole and functionalised pyrrole copolymer, fixed on said material composing the base of said microtroughs.~~

- ✓ 5        20. Biochip comprising in this order:
- a silica substrate,
  - a gold layer or a silane layer comprising pyrrole sites,
  - a resin layer coating the gold layer or silane
- 10 layer comprising pyrrole sites, wherein microtroughs have been produced such that the base of said microtroughs is composed at least partly of said gold layer or said silane layer comprising pyrrole sites,
- a layer of a pyrrole and functionalised pyrrole
- 15 copolymer, fixed on the gold layer or silane layer comprising pyrrole sites composing the base of said microtroughs, the functionalised pyrrole being bound or not to a bi-functional cross-linking agent, et
- an oligonucleotide fixed directly on the
- 20 functionalised pyrrole, or indirectly on the functionalised pyrrole by means of the cross-linking agent bound to said pyrrole.

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